Historic, archived document

Do not assume content reflects current scientific knowledge, policies, or practices.

109

U. S. DEPARTMENT OF AGRICULTURE

OFFICE OF EXPERIMENT STATIONS

BULLETIN No. 18

CHEMICAL DIVISION
LIBRARY
U.S. Dept. of Agr.

A CONTRIBUTION TO THE INVESTIGATION

OF THE

ASSIMILATION OF FREE ATMOSPHERIC NITROGEN

BY

WHITE AND BLACK MUSTARD

BY

J. P. LOTSY, Ph. D.

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE

WASHINGTON
GOVERNMENT PRINTING OFFICE
1894

109

CHEMICAL DIVISION
LIBRARY

U. S. DEPARTMENT OF AGRICULTURE

OFFICE OF EXPERIMENT STATIONS

BULLETIN No. 18

A CONTRIBUTION TO THE INVESTIGATION OF THE ASSIMILATION OF FREE ATMOSPHERIC NITROGEN BY WHITE AND BLACK MUSTARD

BY

J. P. LOTSY, Ph. D.

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE

WASHINGTON
GOVERNMENT PRINTING OFFICE
1894

OFFICE OF EXPERIMENT STATIONS.

A. C. TRUE, Director.

E. W. ALLEN, Assistant Director and Editor of departments of Chemistry, Foods and Animal Production, and Dairying.

W. H. BEAL, Editor of departments of Meteorology, Fertilizers, Soils, and Indexes.

WALTER H. EVANS, Editor of departments of Botany, Seeds, Weeds, and Diseases of Plants.

J. F. DUGGAR, Editor of departments of Field Crops, and Veterinary Science and Practice.

W. O. ATWATER, Special Agent.

F. W. WOLL, Special Agent.

F. H. HALL, Librarian.

S. L. SOMMERS, Record Clerk.

THE AGRICULTURAL EXPERIMENT STATIONS.

ALABAMA—*Auburn:* College Station, W. L. Broun.† *Uniontown:* Canebrake Station; H. Benton.‡

ARIZONA—*Tucson:* F. A. Gulley.*

ARKANSAS—*Fayetteville:* R. L. Bennett.*

CALIFORNIA—*Berkeley:* E. W. Hilgard.*

COLORADO—*Fort Collins:* Alston Ellis.*

CONNECTICUT—*New Haven:* State Station; S. W. Johnson.* *Storrs:* Storrs School Station; W. O. Atwater.*

DELAWARE—*Newark:* A. T. Neale.*

FLORIDA—*Lake City:* O. Clute.*

GEORGIA—*Experiment:* R. J. Redding.*

IDAHO—*Moscow:* C. P. Fox.*

ILLINOIS—*Champaign:* G. E. Morrow.†

INDIANA—*La Fayette:* C. S. Plumb.*

IOWA—*Ames:* James Wilson.*

KANSAS—*Manhattan:* G. T. Fairchild.§

KENTUCKY—*Lexington:* M. A. Scovell.*

LOUISIANA—*Audubon Park, New Orleans:* Sugar Station. *Baton Rouge:* State Station. *Calhoun:* North Louisiana Station. W. C. Stubbs.*

MAINE—*Orono:* W. H. Jordan.*

MARYLAND—*College Park:* R. H. Miller.*

MASSACHUSETTS—*Amherst:* State Station; C. A. Goessmann.* *Amherst:* Hatch Station; H. H. Goodell.*

MICHIGAN—*Agricultural College:* L. G. Gorton.*

MINNESOTA—*St. Anthony Park:* W. M. Liggett.§

MISSISSIPPI—*Agricultural College:* S. M. Tracy.*

MISSOURI—*Columbia:* E. D. Porter.*

MONTANA—*Bozeman:* S. M. Emery.*

NEBRASKA—*Lincoln:* C. L. Ingersoll.*

NEVADA—*Reno:* S. A. Jones.*

NEW HAMPSHIRE—*Durham:* G. H. Whitcher.*

NEW JERSEY—*New Brunswick:* State Station; E. B. Voorhees.* College Station; A. Scott.*

NEW MEXICO—*Las Cruces:* H. Hadley.*

NEW YORK—*Geneva:* State Station; P. Collier.* *Ithaca:* Cornell University Station; I. P. Roberts.*

NORTH CAROLINA—*Raleigh:* H. B. Battle.*

NORTH DAKOTA—*Fargo:* J. B. Power.*

OHIO—*Wooster:* C. E. Thorne.*

OKLAHOMA—*Stillwater:* J. C. Neal.*

OREGON—*Corvallis:* J. M. Bloss.*

PENNSYLVANIA—*State College:* H. P. Armsby.*

RHODE ISLAND—*Kingston:* C. O. Flagg.*

SOUTH CAROLINA—*Fort Hill:* E. B. Craighead.*

SOUTH DAKOTA—*Brookings:* L. McLouth.‖

TENNESSEE—*Knoxville:* C. F. Vanderford.**

TEXAS—*College Station:* J. H. Connell.*

UTAH—*Logan:* J. W. Sanborn.*

VERMONT—*Burlington:* J. L. Hills.*

VIRGINIA—*Blacksburg:* J. M. McBryde.*

WASHINGTON—*Pullman:* E. A. Bryan.

WEST VIRGINIA—*Morgantown:* J. A. Myers.*

WISCONSIN—*Madison:* W. A. Henry.*

WYOMING—*Laramie:* A. A. Johnson.*

---

* Director.

† President of board of direction.

‡ Assistant director in charge.

§ Chairman of council.

‖ Acting director.

** Secretary.

LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
OFFICE OF EXPERIMENT STATIONS,
Washington, D. C., December 16, 1893.

SIR: I transmit herewith for publication as Bulletin No. 18 of this Office an article describing the methods and results of an investigation regarding the assimilation of the free nitrogen of the air by two species of mustard. This investigation has been carried on by J. P. Lotsy, PH. D., Associate in Botany at Johns Hopkins University. There is at present great interest among scientific and practical agriculturists in everything which relates to the problem of the assimilation of atmospheric nitrogen by plants, and it is believed that the careful work reported in this bulletin will be of service, not only because of the results attained, but also by stimulating more thorough and rigorous inquiries on this great question.

Respectfully,

A. C. TRUE,
Director.

Hon. J. STERLING MORTON,
Secretary of Agriculture.

THE ASSIMILATION OF FREE ATMOSPHERIC NITROGEN BY WHITE AND BLACK MUSTARD.

By J. P. Lotsy, Ph. D.

The question whether the higher plants are able to assimilate the free atmospheric nitrogen is a problem which has been treated again and again. Since Boussingault's classical experiments the possibility of an assimilation of free nitrogen by the higher plants has been discarded by nearly all botanists, and only very few of them, like Frank and his associates, claim that most, if not all, plants possess this property. The question is of vital importance from an economic as well as a scientific standpoint. If it could be proved that our present notions of the inability of plants to assimilate free nitrogen were wrong, there would be radical changes in the use of artificial nitrogenous manures. The experiments made by Frank and others, which indicated that plants of different kinds could assimilate free nitrogen, are far from conclusive. Frank himself has never published his methods in sufficient detail to allow of criticism. For this reason the experiments reported in this bulletin were made. Before going into the question more in detail, it is desirable to explain why the mustard species were chosen for these experiments. The reason is that recently in many European agricultural papers it has been suggested that the mustards were able to enrich the soil in nitrogen. Now, experience teaches us, for example, in the case of the assimilation of free nitrogen by the *Papillionaceæ*, that practice very often recognizes facts before science is able to explain them. That others followed about the same course of reasoning becomes clear from the fact that recently, after my work was under way, some papers appeared which gave accounts of experiments with the same plants.

Before describing the experiments it will be well to consider in some detail the methods most frequently used by botanists to determine the assimilation of free nitrogen by plants, since the different methods strictly taken do not answer the same question. Hellriegel and his associates attack the problem of the assimilation of free nitrogen in this way: They begin by keeping away from the plants all sources of fixed nitrogen. The seeds are sprouted in distilled water, cultivated in sand, which has been heated to redness and has been washed with acids, and finally are watered with carefully prepared nitrogen-free food solutions and distilled water. If the plant grows and shows upon chemical analysis more nitrogen in its substance than was contained in the seed the conclusion is drawn that the plant assimilates free nitrogen.

Frank and his associates approach the question in a fundamentally different manner. The seeds are sprouted in the same way, but then the plant is not put in a soil free from nitrogenous salts, but in a soil which possesses a certain known amount of nitrogenous substance. If it is known, for example, that 3 grams of fixed nitrogen were put into the pot, and that the seed contained 0.5 gram, then the nitrogen stock of the plant would be 3+0.5=3.5 grams. If analysis at the end of the experiment shows the presence of 1.5 grams in the soil and 2 grams of fixed nitrogen in the crop, 1.5+2=3.5 grams will be the total fixed nitrogen present, or the same amount as in the beginning of the experiment. It is thus made to appear that the plant had no need of an assimilation of free nitrogen. Whether it did or not remains to be seen, as fixed nitrogen present at the beginning of the experiment may have been decomposed, and free nitrogen fixed by the plant added. If, on the other hand, the soil contained 3 grams and the crop 2.5 grams, 5.5 grams would be the total fixed nitrogen at the end of the experiment. The 2 grams of additional fixed nitrogen can only have come from the atmosphere.

In the method used by Schlösing, the plant is cultivated in an air-tight glass chamber into which air free of ammonia is introduced. By analyzing this air before and after the growth of the plant, it can be determined whether there is a decrease in the amount of nitrogen or not. This method is by far the most direct one, but very great difficulties are encountered in carrying out the experiments.

Now let us see what different questions are answered by these methods strictly taken. The first method simply shows whether the plant is able to take *all* the nitrogen it needs from the atmosphere. The second method gives no results whatever in case the crop has no more nitrogen than was contained in the seed and soil; but in case there is an increase, it shows that the plant is able to supply itself partly with free nitrogen, but does not indicate whether the plant can live on free nitrogen exclusively, as it has some fixed nitrogen to start with. The third method may answer both questions. This will depend on the existence or not of fixed nitrogen in the soil at the beginning of the experiment. Even this method is, however, not entirely free from objections, on account of the fact that the plants have to be cultivated under unusual conditions, owing to the limited space in which they grow.

To get a clear insight into the question of the assimilation of free nitrogen, there remains conclusively but one thing to be done, viz, apply all three methods to the same kind of plant in each case. It is, in fact, possible that a plant may only be able to assimilate free nitrogen, if fixed nitrogen is present, or that at least only under those circumstances (Frank even claims this) is the assimilation of free nitrogen of practical value. It is furthermore possible that the plant may need fixed nitrogen during a certain period of its life, say during the young stage* as a seedling.

Not even after we have applied all three methods to the same plant and have invariably found that the plant assimilates free nitrogen, are we sure that our interpretation of the facts is correct. Microörganisms present in the culture media may have assimilated the free nitrogen, and the plants may have used this fixed nitrogen which originated entirely without their aid. The only way to overcome all difficulties is to grow plants by all three methods, using two parallel series in each case, one sterilized and the other unsterilized. This is tedious work, no doubt, but it is the only way to come to a well-founded conclusion.

The publication of accounts of certain recent experiments on the free assimilation of atmospheric nitrogen has made it advisable to issue this bulletin before tests by all three methods were completed.

Liebscher (*Journal für Landwirthschaft, 1893*) has published a report on some investigations carried on by the second method, in which it appeared that the mustard assimilated free nitrogen. The experiments here reported gave an opposite result, which, however, does not interfere directly with Liebscher's results, as his experiments do not answer the same question as those of the author. Liebscher's experiments have received much attention from agriculturists but need confirmation before field experiments on a large scale are attempted. His methods were not as rigorous as is desirable. For example, his plants were not watered with distilled water absolutely free from nitrogen, but with hydrant water which he himself admits contained some nitrogen.

Two articles by B. Frank (*Deutsche Landw. Presse, 1893; Botanische Zeitung, Sept. 16, 1893*, in detail) concern us more particularly. Frank claims that plants cultivated in sand absolutely free from fixed nitrogen show an increase in nitrogen, though very much less than when fixed nitrogen is present in the soil. He admits that this increase is insignificant in the case of non-leguminous plants. For potatoes (*Solanum tuberosum*) he found an increase from 0.022 to 0.2186 gram (about tenfold) in case no fixed nitrogen was present in the soil. White mustard showed an increase from 0.0012 to 0.0043 gram, or about 3½ fold, in the absence of fixed nitrogen, as compared with 350 fold when nitrogen was present in the soil.

Frank does not publish his method in detail, but mentions the fact that, in most cases at least, his cultures were not sterilized. In such cases the fixation of nitrogen was probably due to microörganisms.

In the experiments reported in this bulletin it will appear that the

---

*After this was written, Frank's article appeared in *Botanische Zeitung*, 1893, where he says (p. 153) that the plants, with the exception of the *Leguminosæ*, need nitrates during their young stage, and only afterwards are able to assimilate free nitrogen.

black and white mustards do not assimilate free nitrogen in the absence of fixed nitrogen, and that therefore Frank is wrong in claiming that these plants do assimilate perceptible quantities of free nitrogen, even when nitrates are not present in the soil.

As the trustworthiness of the results of investigations in this line depends entirely on the method employed, the experiments here reported will be described in detail.

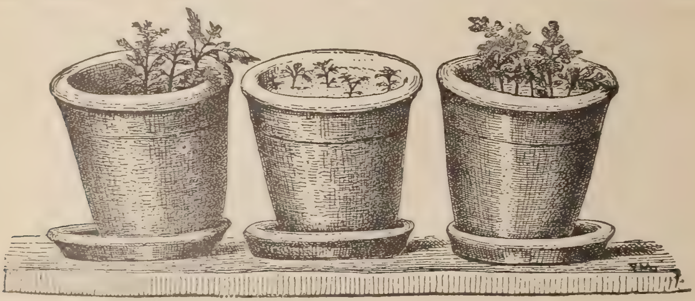

FIG. 1.—Sand cultures of *Sinapis alba*, July 31, with nitrates.

For the experiment eight rows of cultures were used. The first row contained 6 sand cultures, 3 with white and 3 with black mustard, which received nitrates as a fertilizer. The second row also contained 6 sand cultures, 3 with white and 3 with black mustard, treated in every respect like the first row, except that they received no nitrates. The third row contained 6 water cultures, 3 of white and 3 of black mustard, which received nitrates. The fourth row was the same as the third, except that no nitrates were used. These four rows were not

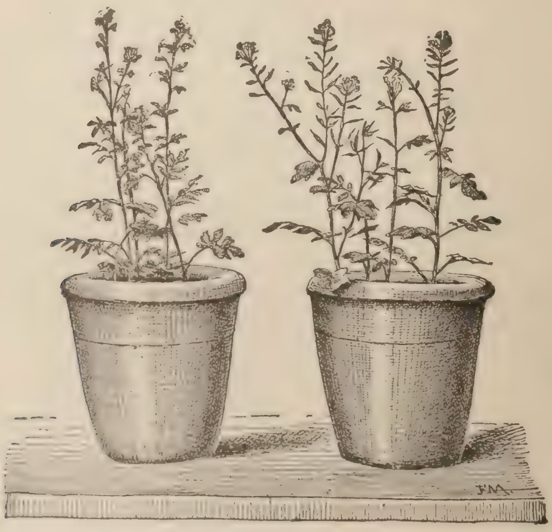

FIG. 2.—Sand cultures of *Sinapis alba*, Aug. 30, with nitrates.

sterilized, so that microörganisms were allowed to develop. The fifth row contained 4 sand cultures, 2 with white and 2 with black mustard, which were treated like those in the first row, except that they were sterilized. The sixth row contained 4 sand cultures like those of the second row, but sterilized. The seventh row contained 4 water cultures like those in the third row, but sterilized. The eighth row was identical with the fourth row except that the cultures were sterilized. In the last four rows of cultures the action of microörganisms was excluded.

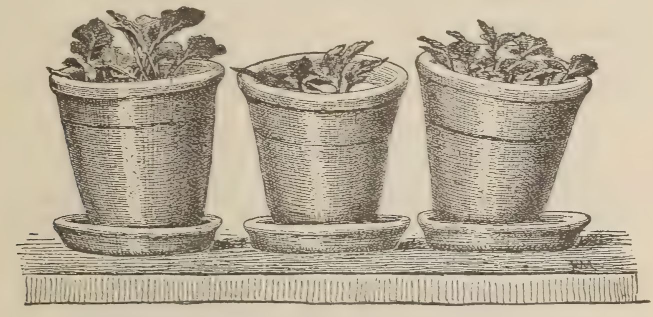
FIG. 3.—Sand cultures of *Sinapis nigra*, July 31, with nitrates.

All chemicals used in the experiments were of the grade "Purissimum" and yet were first tested for the possible presence of nitrogen. The calcium sulphate which could not be obtained here in that grade was imported from E. Merck, of Darmstadt, Germany. The water used for watering the plants as well as that for preparing the nutritive solutions, was redistilled until it failed to give the faintest blue coloration with diphenylamin or reddish coloration with Nessler's reagent. For the pots the finest sand obtainable in the neighborhood was used. This

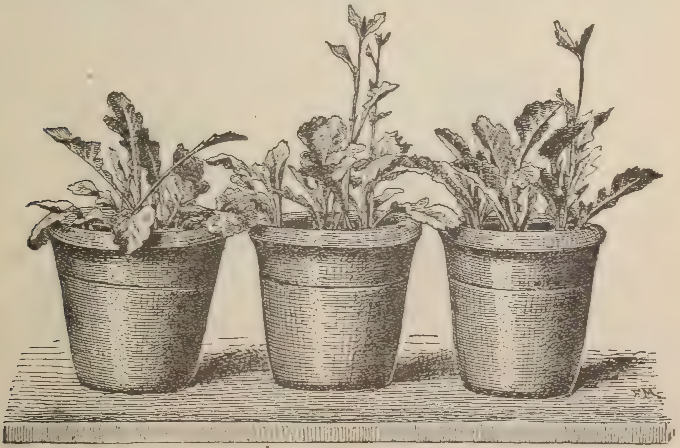
FIG. 4.—Sand cultures of *Sinapis nigra*, Aug. 30, with nitrates.

was washed under a constant stream of hydrant water to eliminate all particles of clay and substances soluble in water, and this process was continued until the water, under constant stirring of the sand, ran away perfectly clear. Four quarts of sand were washed at a time and as the stream of hydrant water ran constantly for about three hours it was thoroughly cleansed.

After this, 3 quarts of 20 per cent chemically pure hydrochloric acid tested for nitrogen was added. In this the sand remained for twenty-four hours and subsequently was washed with distilled water until it did not give any acid reaction with litmus. This took for 4 quarts of sand about four or five hours and about 50 quarts of distilled water was used for the purpose. The sand was then dried in the air bath at a temperature of about 140° C., and afterwards ignited in a Hessian fire-proof crucible. The pots were soaked in distilled water for twenty-four hours and subsequently rinsed out with distilled water several times.

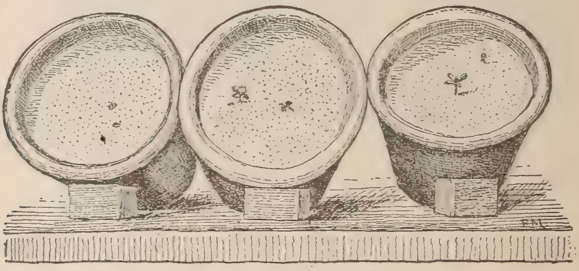

Fig. 5.—Sand cultures of *Sinapis alba*, July 31, without nitrates.

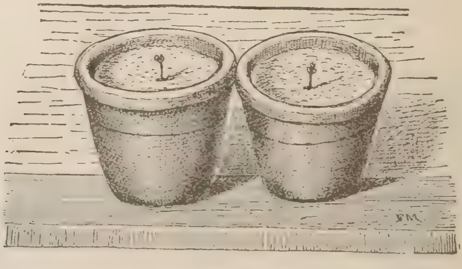

Fig. 6.—Sand cultures of *Sinapis alba*, Aug. 30, without nitrates.

The sand cultures in the first series were made as follows: The flower pots used were ordinary porous pots, each of which contained about 2½ quarts of sand. This series was not sterilized and received the necessary fertilizer by watering with a nutritive solution containing 2 parts of nitrate of potassium, 1 part of chloride of sodium, 1 part of tricalcium phosphate, 1 part of calcium sulphate, 1 part of magnesium sulphate, and a few drops of chloride of iron. The concentration of the solution was 3:1,000 (0.3 per cent). In this way six pots were treated. The seeds necessary for this experiment were sprouted in distilled water on filter paper. In each pot five seedlings a few days old were planted. In three pots (Figs. 1 and 2) *Sinapis alba* was planted, in the other three (Figs. 3 and 4) *S. nigra*. The plants were put in July 19, and were placed, in the absence of a hothouse, on the roof of the biological building of the university, where they were protected from too strong sunlight by screens of cheese cloth. The plants grew excellently, and throughout the experiment, which ended August 30, not a plant was lost. Pot 2 of *Sinapis alba* was blown over during a severe windstorm and had to be repotted, which of course kept the plants back in their development as seen in Fig. 1. This pot is not shown in the illustration taken at the end of the experiment, as the plants were yet backward in their development. All plants bore flowers, fruits, and seeds in abundance. Six other pots were treated in the same manner, except that the two parts of nitrate of potassium were replaced by one part of chloride of potassium at the beginning of the experiment, and one part applied later on. Consequently these plants had no other nitrogen than the free nitrogen of the atmosphere. During rainy weather glass plates were put over the tops of the pots to keep them from receiving the traces of HNO_3 in the rain. As there was hardly any rain during the whole period of the experiment, there was practically no error from this cause. Of these pots, pot 1 containing *Sinapis alba*

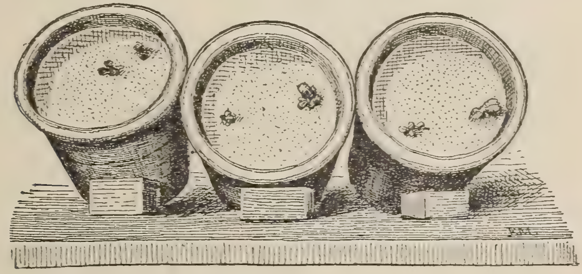

FIG. 7.—Sand cultures of *Sinapis nigra*, July 31, without nitrates.

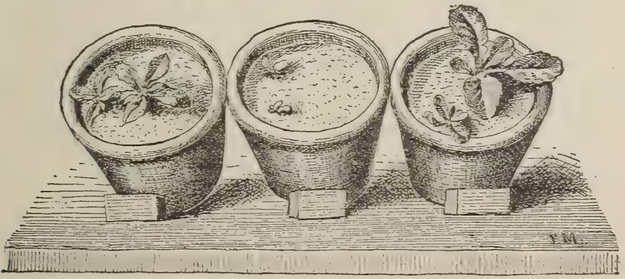

FIG. 8.—Sand cultures of *Sinapis nigra*, Aug. 30, without nitrates.

showed July 31 (Fig. 5) but one very small and weak plant, pot 2 had two, and pot 3, one. August 30 (Fig. 6) pot 1 had one tiny plant, pot 2 none at all, and pot 3 only a very weak one. None of these plants, however, were larger than was to be expected considering the accumulated nitrogen present in the seed. In the case of *Sinapis nigra*, each of the three pots showed on July 31 (Fig. 7) two small plants. August 30 (Fig. 8) pots 1 and 3 had two unexpectedly large plants, considering the circumstances. Pot 2 had two small plants. None of these plants, however, had attained the size and vigor of those which received nitrates. We see here how necessary it is to make more than one series of experiments, for we would have concluded from this solitary series that *Sinapis nigra* could get along at least in a poor way without fixed nitrogen. On the pots there was a vegetation of algæ and it is just possible that these assimilated the free nitrogen, as indicated by the experiments of Frank and Schlösing. Beyerinck, however, was not able to demonstrate this property with those algæ which he could get in pure cultures. Frank and Schlösing worked with impure cultures in which other organisms may have played a role.

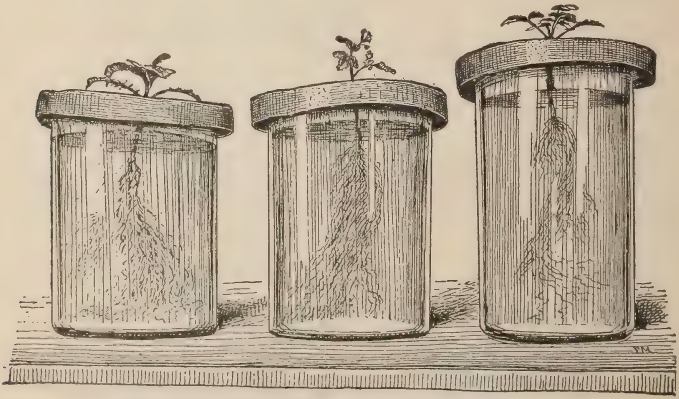

FIG. 9.—Water cultures of *Sinapis alba*, July 31, with nitrates.

It is also possible, but very improbable, that the small quantity of rain which the pots received contained a sufficient amount of nitrogen for *Sinapis nigra* but not for *Sinapis alba*. As there was a possibility that the soil might accumulate fixed nitrogen without the action of the mustard plants to every series of pots one pot was added which did not contain any plants at all, but which was treated in all respects exactly like those which did have plants. These pots also showed an accumulation of fixed nitrogen.

It was interesting to observe that the *Sinapis nigra* grew much better than *Sinapis alba*, notwithstanding all the plants were exposed to exactly the same influence, so that it would be well-nigh miraculous if the pots with *Sinapis nigra* had accumulated more nitrogen than those with *Sinapis alba*. We have therefore to conclude that *Sinapis nigra* is able to live on less fixed nitrogen than *Sinapis alba*. This is remarkable in plants so closely allied and it might be of practical value to determine by exact experiments how different plants behave in this respect.

The illustrations are from drawings made from photographs by the author. All the photographs were taken at the same distance, and great pains taken to make the drawings of the same size as the photographs. The exact relations as to size, etc., which existed between the different plants are therefore shown in the illustrations.

To recapitulate: *We see from the illustrations that the plants which received fixed nitrogen grew regularly from July 19 to August 30, after which date they formed seed. Those which received no fixed nitrogen grew a little till July 31 and the combined quantity of produced organic matter was at the end of the experiment even a little less than on July 31, owing to the death of one of the plants caused by nitrogen hunger. It is noticeable, however, that the plants of black mustard which received no fixed nitrogen grew towards the end of the experiment considerably more than those of white mustard.*

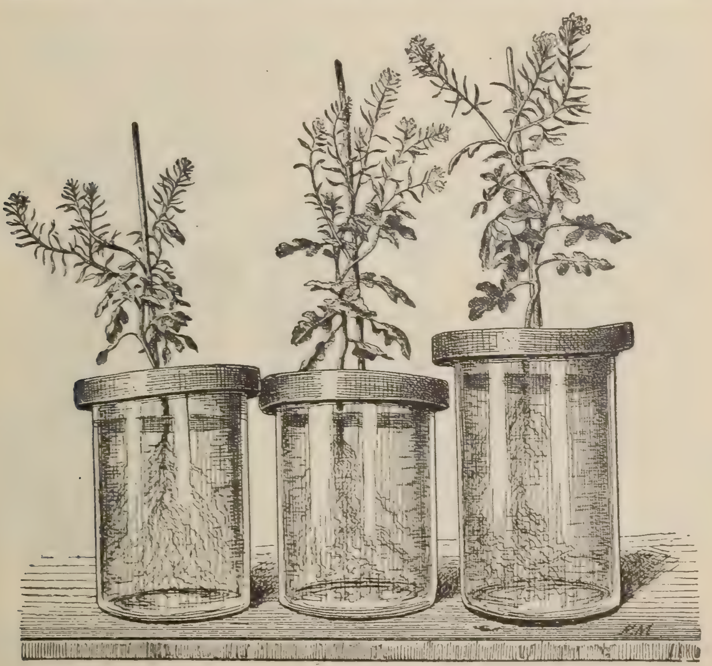

FIG. 10.—Water cultures of *Sinapis alba*, Aug. 30, with nitrates.

Now let us see how these plants behaved in water cultures. For this series six glass vessels were used which were scrupulously cleaned and afterwards filled with the same nutritive solutions as those which were used for sand cultures. The concentration was at first made 3 per cent and during the whole course of the experiment the used-up food solution was replaced by distilled water until the original volume was reached. The vessels contained about 6 quarts and to every quart 3 drops of a concentrated aqueous solution of chloride of iron were added. The part of the culture vessel containing the roots was protected from the light by means of paper. On top of the vessel was placed a wooden cover with a round hole in the center in which the seedling was fixed by means of cotton. In three of the vessels *Sinapis alba* (Figs. 9 and 10) was planted and in the three others *Sinapis nigra* (Figs. 11 and 12). All these plants developed excellently.

Six other vessels were treated exactly in the same way, but in place of two parts of nitrate of potassium, one part of chloride of potassium, later on increased to two parts, was used. All these plants died before August 1 (Fig. 13), after having developed but very little.

To recapitulate: *All plants which received fixed nitrogen were normally and completely developed both in sand and in water cultures. Of those which got no nitrogen, all in water cultures died, while the sand cultures of* Sinapis alba *did not show any more development than could be explained by the nitrogen contained in the seed. The sand cultures of* Sinapis nigra, *however, developed so far as to indicate an assimilation of free nitrogen.*

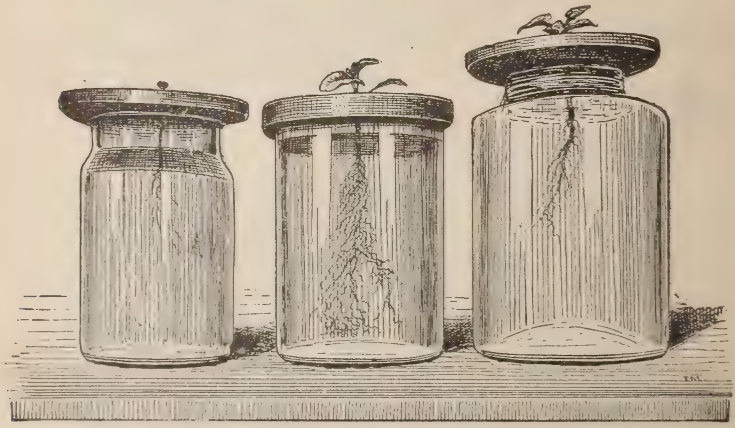

FIG. 11.—Water cultures of *Sinapis nigra*, July 31, with nitrates.

To find out whether the plants themselves were the cause of these phenomena or whether other influences, *e. g.*, the action of microörganisms, played a role here, a series of sterilized sand and water cultures were made.

The sterilized sand cultures were prepared as follows: The pots were glazed on the outside and the hole in the bottom was plugged up with cotton. They were then filled with the prepared sand. Subsequently a powder containing 3 grams either of the complete nutritive material or of the one in which the nitrate of potassium was replaced by the chloride was added to every pot. These powders had previously been sterilized by exposing them in glass vessels for five hours to a dry heat at a temperature of 140° C., every 3 grams being separately wrapped up in paper. After this the top of the pot was closed by tying over it a double sheet of cotton. The pots were then placed in the dry sterilizer together with the following articles: (1) One glass vessel about 50 cm. high, the bottom of which was thickly covered with cotton and the opening at the top closed by a double sheet of cotton tied over it; (2) a long straight glass tube, plugged at both ends with cotton; (3) two small U-shaped glass tubes with cotton plugged openings; (4) a glass bottle closed with a rubber stopper with two openings and covered with a double sheet of cotton. All these were exposed during five hours to a temperature of 140° C. After cooling, the pot was placed inside of the glass vessel on the cotton, which held it firmly in position. The cotton tied over the top of the pot was taken away and the top of the glass vessel was again closed with a double sheet of sterilized cotton. In this a small hole was made through which the long tube was inserted, so that its lower end rested on the sand in the pot while the upper end extended about 1 cm. outside of the vessel above the cotton. Over all a piece of parchment paper was tied with a hole in it for the tube and where this was too big it was again closed by means of a little wadding. Distilled water was put in the bottle and the two small glass tubes were put in the stopper after the manner of a wash bottle. In one end of the shorter tube a little plug of cotton remained. The tube which reached the water was now united with the tube in the glass vessel by means of a rubber tube. Finally cotton was tied around the stopper of the water bottle and also around the places where the

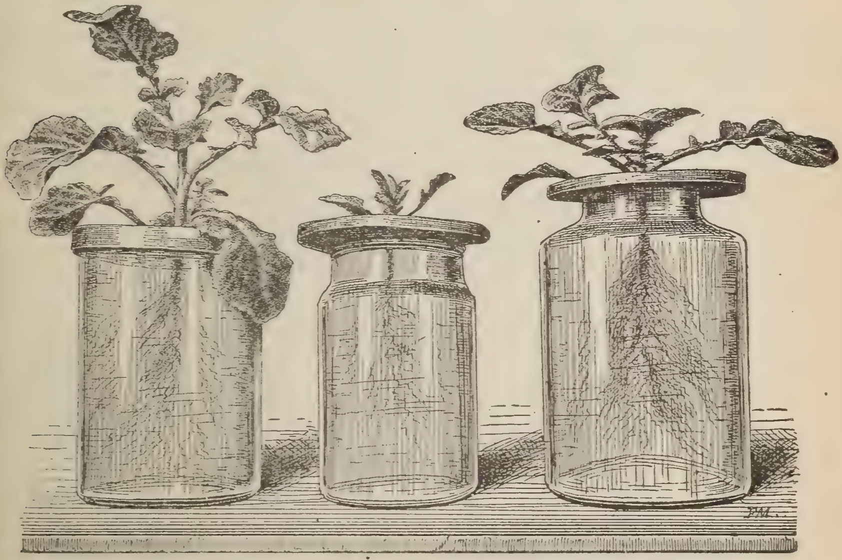

FIG. 12.—Water cultures of *Sinapis nigra*, Aug. 30, with nitrates.

rubber tubing fitted around the glass tubes. The whole apparatus put together in the manner described was now sterilized in the steam sterilizer on three consecutive days for three quarters of an hour each day. For this an ordinary steam sterilizer was used to which an elongation had been attached which could easily be detached. This piece was taken off and the culture vessel placed in the sterilizer, an assistant holding the water bottle. The elongation piece was then put over the water bottle and while the experimenter grasped the bottle from above the assistant put the elongation piece into position again. The water bottle was now suspended by means of a copper wire tied around its neck and fixed to the upper border of the elongation piece. Subsequently the top was put on and sterilization allowed to take place. The arrangement of the culture is shown in Fig. 14.

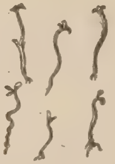

Fig. 13.—Water cultures of *Sinapis alba* and *S. nigra*, July 31, without nitrates.

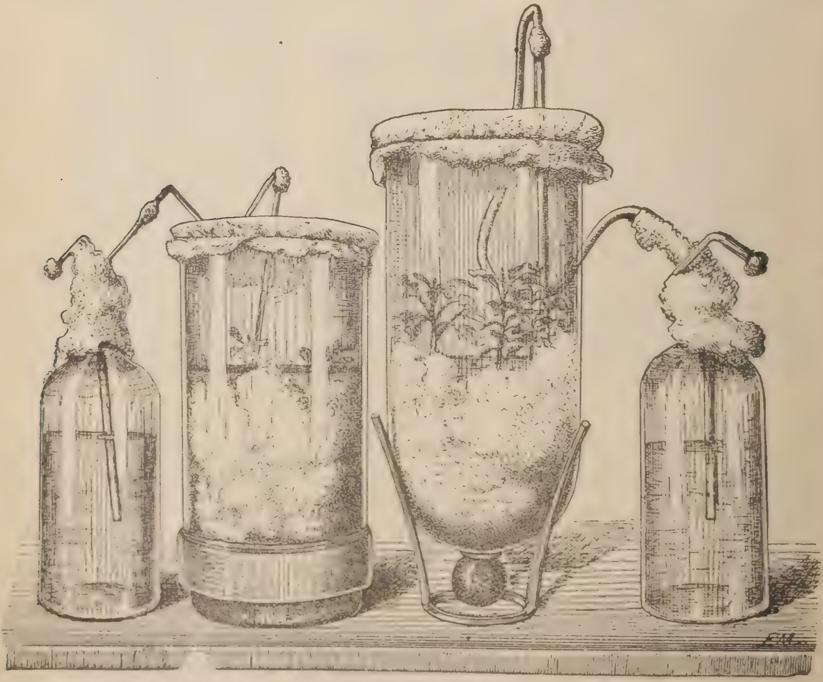

Fig. 14.—Sterilized sand cultures of *Sinapis alba* and *S. nigra*, August 30, with nitrates.

The seeds used in these experiments were first washed with corrosive sublimate of a concentration of 1:1,000. This had to be done quite rapidly, as it was found that the seeds were easily killed by the action of the sublimate. After that they were exposed, inclosed in sterilized petri-dishes during two days, to very strong sunlight to kill the bacteria as far as possible. The layer of seeds was only one thick and they were shaken from time to time to expose all sides equally. During the preliminary experiments it was found that even without the previous washing in sublimate the germinating power was very much decreased by the action of the sunlight, so that seeds apparently behave under such conditions like spores of bacteria. It is not positive that all bacteria were killed by the process, but it was impossible to do more. It was noted that the sprouting seeds treated in this way were never accompanied by fungi as were those which were not treated. It would perhaps be possible to get seeds entirely free from bacteria by cutting them out of the unopened ovary. After having been thus exposed to the action of the sun the seeds were taken from the petri-dishes with sterilized forceps and allowed to sprout on sterilized wet sand in sterilized glass boxes. After they were well sprouted they were taken out by means of sterilized forceps and planted in the following way: Above the glass vessel containing the pot was hung a large inverted glass funnel, the tube of which was plugged with cotton and the inner side wiped out by means of a towel soaked in corrosive sublimate. This served to prevent bacteria from falling into the culture vessel after lifting the parchment paper and cotton. Subsequently the right hand and bare arm of the experimenter were scrubbed with soap and water and washed with sublimate. The cotton parchment cover was lifted just enough to allow the hand to pass through it and the seedlings were planted with sterilized forceps. Five seedlings were planted in each pot. After this all the apparatus was carefully closed again.

By lifting the water bottle and turning it upside down it was now possible to water the plants without danger of contamination. An objection to cultures like this is that the plants are growing in an atmosphere which is very rich in watery vapor, but it is not feasible to avoid this difficulty. Through lack of a sufficient number of large glass vessels only four cultures could be made, two with and two without fixed nitrogen. Five plants of *Sinapis alba* and five of *Sinapis nigra* were planted in each series.

The experiments were begun August 9 and closed August 30, before the plants bloomed. In the cultures which received nitrates both *Sinapis alba* and *Sinapis nigra* developed finely. Though no further attention was paid to them, by September 25 the plants had grown up so as to reach the tops of the vessel and were in bud. At this time the plants were destroyed. According to Frank's experiments it would be impossible to get them to open their buds in such an atmosphere. Those plants which got no nitrates died very soon after planting.

The following apparatus was used in the sterilized water cultures (Fig. 15): Six glass vessels, the openings of which were tied with cotton; six bell glasses, with openings at both sides, both closed with cotton; six straight glass tubes, cotton plugged at both ends; six washing bottles, with cotton tied around the stopper and tubes plugged with cotton, and six corks with holes in the center inclosed in a cotton-plugged glass cylinder. These articles were sterilized during five hours dry at a temperature of 140° C.

Subsequently, the washing bottles were filled with sterilized distilled water; three of the vessels were filled with sterilized nutritive solutions containing nitrates, and the three others with similar solutions, in which the nitrate was replaced by chloride of potassium.

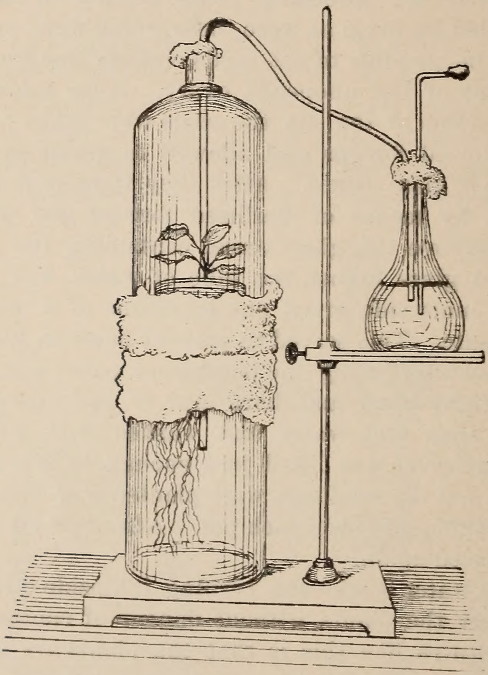

Fig. 15.—Sterilized water culture of *Sinapis nigra*, August 30, with nitrates.

After this cotton was wound around the mouth of the glass vessels and bell jars placed above the glass vessels in such a way that the joints were tightly closed by the cotton, the remaining openings being plugged with cotton. Around the joints, between the glass vessels and bell jars, a sheet of cotton was wound and tied above and below the rim of the bell jar. The glass tube was introduced into the food solution through the upper opening of the bell glass, the cotton plug was replaced, and the end of the tube projecting from the bell jar was united with the tube which reached the water in the wash bottle by means of a rubber tube. Cotton was tied around the places where the rubber touched glass. The whole apparatus, put together in this way, was sterilized in the manner described for the sand cultures. The seeds were prepared in the same way as those for the sand cultures. The seedlings were fixed by means of sterilized cotton in the corks which just before were passed through the flame. By lifting the bell glass a little it was now possible to put corks and plants into the culture vessel and let them float on the food solution. After having replaced the outer sheet of cotton a piece of paper was tied around the under half of the apparatus to prevent the light from doing harm to the root system of the plant. Four of the vessels were filled with food solutions free of nitrates and two received nitrates. The two plants in the food solution with nitrates grew very well (see Fig. 15), the four without nitrates died.

The conclusion to which these experiments lead us is this: *Neither of the two mustard species,* Sinapis alba *and* S. nigra, *is able to live without fixed nitrogen.*